Kinesins Lead Aging Microtubules to Catastrophe

Anna Akhmanova[1,*] and Marileen Dogterom[2,*]
[1]Division of Cell Biology, Faculty of Science, Utrecht University, Padualaan 8, 3584 CH, Utrecht, The Netherlands
[2]FOM Institute for Atomic and Molecular Physics (AMOLF), Science Park 104, 1098 XG Amsterdam, The Netherlands
*Correspondence: a.akhmanova@uu.nl (A.A.), dogterom@amolf.nl (M.D.)
DOI 10.1016/j.cell.2011.11.011

The ability of growing microtubules to undergo catastrophes—abrupt switches from growth to shortening—is one of the key aspects of microtubule dynamics important for shaping cellular microtubule arrays. Gardner et al. show that catastrophes occur at a microtubule age-dependent rate and that depolymerizing kinesins can affect this process in fundamentally different ways.

Microtubules are polymeric filaments built from dimers of α- and β-tubulin. Tubulin dimers attach to each other in a head-to-tail fashion to form protofilaments, which in turn interact laterally to shape a hollow tube, the microtubule. Microtubule ends are structurally and functionally distinct: microtubule minus ends grow slowly in solutions of purified tubulin but never grow in vivo, whereas the plus ends grow rapidly in vitro and represent the sites of microtubule elongation in cells. Importantly, microtubule plus ends can abruptly switch from growth to depolymerization (an event called catastrophe) and also undergo reverse transitions (rescues). This behavior, termed dynamic instability, can be observed both in vitro and in cells. One of the key questions in microtubule biology is how the different phases of dynamic instability are regulated by different cellular factors. The study by Gardner et al. (2011) in this issue of *Cell* sheds new light on the intrinsic mechanism of microtubule catastrophes and their control by the members of two kinesin families, kinesin-8 and kinesin-13.

At first glance, microtubule catastrophe appears to be a random process, limited by a single rate-limiting event. This step is commonly believed to be associated with the random loss of a "GTP cap." Tubulin is incorporated into growing filaments in complex with GTP. Subsequent hydrolysis to GDP drives tubulin into its mature form within the microtubule lattice. The GTP cap represents the layer of tubulin subunits in which the β-tubulin-associated GTP has not yet been hydrolyzed. Consistent with a single rate-limiting step for catastrophe, early in vitro measurements of the distribution of microtubule lengths revealed an apparent exponential distribution (Fygenson et al., 1994). On closer inspection however, Odde et al. (1995) noted that there appear to be fewer short catastrophe times than would be consistent with such a simple picture. An attractive explanation for this is that there are multiple rate-limiting steps, which would predict a peak in the distribution of catastrophe times instead of a simple decaying exponential distribution. Because the difference between these two types of distributions is most striking for early events (i.e., short microtubules), distinguishing between the two scenarios critically depends on the experimental ability to detect very short microtubules. These measurements were difficult in early studies based on microtubule detection with differential interference contrast microscopy. In the current work, the use of double-color total internal reflection fluorescence microscopy eliminated this potential problem, establishing firmly that in vitro microtubules do not experience single-step catastrophe events, at least not at early stages of polymerization.

Fitting of both the earlier and new data was shown to be consistent with a process that involves three rate-limiting steps (Gardner et al., 2011; Odde et al., 1995). The authors therefore propose that a microtubule needs to "age" by experiencing two independent random events before a third random event can trigger a catastrophe (Figure 1A). Importantly, although "young age" of microtubules generally correlates with a short microtubule length, by varying tubulin concentration, the authors convincingly show that it is the age rather than the length that is an important factor determining catastrophe occurrence.

Having described catastrophes in their system in the absence of additional factors, the authors then proceed to test how representatives of the two evolutionary conserved microtubule-destabilizing kinesin families affect catastrophe time distributions. These kinesins promote removal of tubulin dimers from filament ends. Kinesin-8 (represented by Kip3 in budding yeast) has an N terminally located motor domain; it can walk processively along microtubules toward the plus ends. Long microtubules are expected to accumulate kinesin-8s at their tips, and consistently, the authors found that Kip3 can slow down the microtubule growth rate in a microtubule length-dependent manner. Interestingly, in the presence of Kip3, catastrophes were still microtubule age dependent, consistent with a three-step process, but the aging occurred faster (Figure 1B).

The kinesin-13 family (represented by mammalian MCAK) has the motor domain in the middle of the protein. It arrives at microtubule tips through lateral diffusion or interaction with other proteins. The authors showed that, in the presence of MCAK, the microtubule growth rate is not affected, consistent with previously published data (Kinoshita et al., 2001), but microtubule catastrophes become age independent. Intriguingly, the fitted

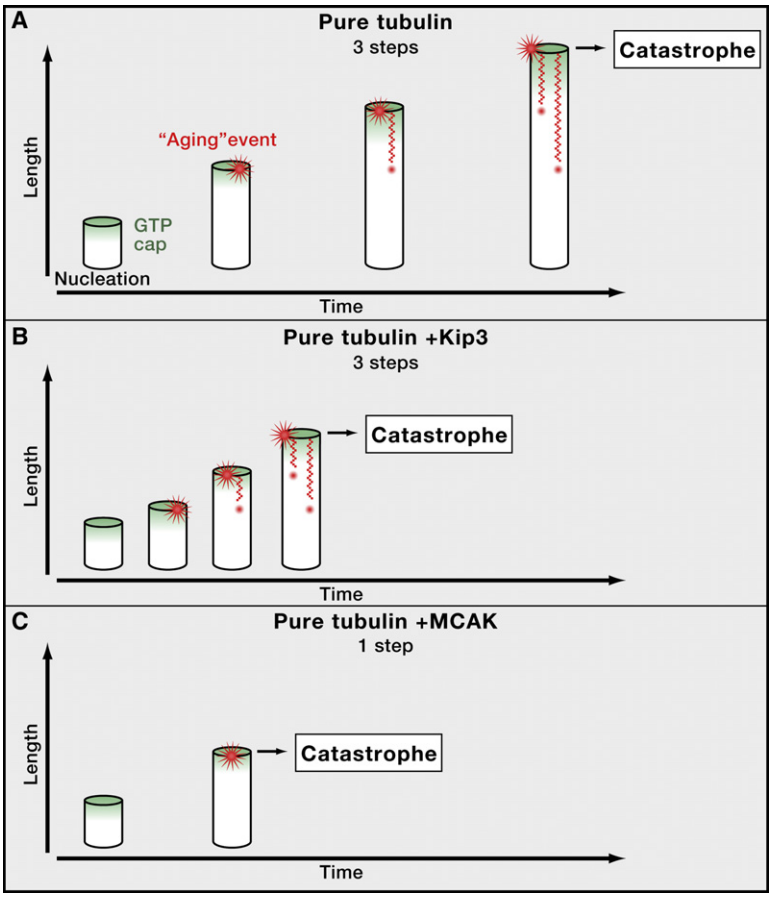

Figure 1. Model for Kinesin Regulation of Microtubule Catastrophe

The model is based on a theoretical analysis of microtubule catastrophe times that gives both the number and rate of "aging" events.

(A) In solutions of pure tubulin, three events (termed "aging events") randomly distributed over time need to occur before a microtubule undergoes a catastrophe. The nature of these events is currently unclear: they may represent loss of the GTP cap from individual protofilaments, loss of a protofilament, or another type of a lattice defect, which is propagated to the microtubule tip as the microtubule keeps growing.

(B) In the presence of kinesin-8 Kip3, microtubule aging preceding catastrophe still occurs in three steps, but the steps occur more quickly.

(C) In the presence of kinesin-13 MCAK, microtubule "aging" can no longer be observed because catastrophe becomes a random single-step process.

single-step rate constant remained similar to the rate of individual aging events for pure tubulin (Figure 1C). Apparently, MCAK eliminated the need for the remaining two steps.

Measurement of microtubule dynamics in neurons and fission yeast indicates that catastrophes are microtubule age related in vivo and can be affected by proteins that bind to microtubule ends (Stepanova et al., 2010; Tischer et al., 2009). Therefore, although the conclusions of Gardner et al. are based on data obtained in vitro, they are highly relevant for understanding how depolymerizing kinesins control the size and density of microtubule arrays in cells. As the authors show, MCAK widens the catastrophe length range and is thus well suited for restructuring microtubule networks, whereas Kip3 narrows the catastrophe length distribution, a property that is important for controlling the length of microtubule arrays.

It is important to note that all experiments by Gardner et al. (2011) were carried out in conditions that precluded the possibility of rescue, whereas in cells, rescues will strongly affect the final shape of microtubule arrays. Furthermore, in the paper, the two kinesins were explored separately, but in cells, kinesin-8s and kinesin-13s can act together, and this link sometimes can be very intimate: mammalian kinesin-8 KIF18B directly binds to MCAK and is required for robust MCAK activity in the mitotic spindle (Tanenbaum et al., 2011).

The results of Gardner et al. have a number of exciting implications. The interesting consequence of taking the three-step picture literally is that somehow individual (structural) events that occur more or less evenly distributed over the lifetime of a microtubule need to be propagated to the microtubule plus end (see Figure 1). It thus becomes a challenge for the future to unravel what these events might be, to find their signs in the microtubule structure, and to understand how they might be affected by depolymerizing kinesins and other microtubule tip-interacting proteins.

Another remaining question concerns the relationship between the catastrophe rate and the growth rate of microtubules. In a simple three-step scenario, microtubule aging would not depend on the growth rate and thus would be the same at different tubulin concentrations. Indeed, raising the tubulin concentration, the authors found only a weak reduction of the catastrophe rate. However, earlier work has shown that this does not hold true for higher tubulin concentrations (Janson et al., 2003; Walker et al., 1988). In addition, previous measurements of the catastrophe time distribution of stalled microtubules were consistent with a much larger (~10) number of steps (Janson et al., 2003).

It will therefore be interesting to see whether other scenarios for microtubule catastrophes can be fitted to these new data as well. For the case of kinesin-8, it is known that motors accumulate at microtubule ends in a length-dependent way, which may lead to a length-dependent catastrophe rate independent of any microtubule aging process (Brun et al., 2009; Tischer et al., 2009). For pure tubulin, another recent model is based on the idea that individual protofilaments contain two to three terminal GTP-associated tubulins that need to be hydrolyzed before catastrophes occur. This model fits with the reported dependence on growth rate at high tubulin concentrations but appears to predict a nonexponential catastrophe time

distribution only for very low growth rates (Brun et al., 2009).

Clearly, a complete understanding of microtubule regulation in vivo will require increasing the complexity in the in vitro reconstitution experiments, as well as a further refinement of modeling approaches.

ACKNOWLEDGMENTS

A.A. is supported by the Netherlands Organization for Scientific Research (NWO-ALW VICI), Netherlands Organization for Health Research and Development (ZonMw-TOP), and Human Frontier Science Program grant (HSFP). M.D. is supported by a NWO-ALW VICI grant and several grants from the "Stichting voor Fundamenteel Onderzoek der Materie (FOM)," which is financially supported by NWO.

REFERENCES

Brun, L., Rupp, B., Ward, J.J., and Nédélec, F. (2009). Proc. Natl. Acad. Sci. USA *106*, 21173–21178.

Fygenson, D.K., Braun, E., and Libchaber, A. (1994). Phys. Rev. E Stat. Phys. Plasmas Fluids Relat. Interdiscip. Topics *50*, 1579–1588.

Gardner, M.K., Zanic, M., Gell, C., Bormuth, V., and Howard, J. (2011). Cell *147*, this issue, 1092–1103.

Janson, M.E., de Dood, M.E., and Dogterom, M. (2003). J. Cell Biol. *161*, 1029–1034.

Kinoshita, K., Arnal, I., Desai, A., Drechsel, D.N., and Hyman, A.A. (2001). Science *294*, 1340–1343.

Odde, D.J., Cassimeris, L., and Buettner, H.M. (1995). Biophys. J. *69*, 796–802.

Stepanova, T., Smal, I., van Haren, J., Akinci, U., Liu, Z., Miedema, M., Limpens, R., van Ham, M., van der Reijden, M., Poot, R., et al. (2010). Curr. Biol. *20*, 1023–1028.

Tanenbaum, M.E., Macurek, L., van der Vaart, B., Galli, M., Akhmanova, A., and Medema, R.H. (2011). Curr. Biol. *21*, 1356–1365.

Tischer, C., Brunner, D., and Dogterom, M. (2009). Mol. Syst. Biol. *5*, 250.

Walker, R.A., O'Brien, E.T., Pryer, N.K., Soboeiro, M.F., Voter, W.A., Erickson, H.P., and Salmon, E.D. (1988). J. Cell Biol. *107*, 1437–1448.

Contribution of I_h to LTP, Place Cells, and Grid Cells

Mayank R. Mehta[1,*]

[1]Keck Center for Neurophysics, Department of Physics and Astronomy, Department of Neurology, Department of Neurobiology, Integrative Center for Learning and Memory, University of California, Los Angeles, Los Angeles, CA 90095, USA

*Correspondence: mayankmehta@ucla.edu

DOI 10.1016/j.cell.2011.11.005

The brain's grid and place cells, which contribute to spatial representations of the external environment, are thought to be modulated by the hyperpolarization-activated cation current (I_h). Giocomo et al. and Hussaini et al. now provide new insights into these cells' unique activity patterns by studying transgenic mice lacking I_h.

During spatial exploration, hippocampal neurons, called place cells, display spatially selective activity. Upstream of place cells are grid cells of the medial entorhinal cortex (mEC), which are active in multiple place fields that are distributed in a spatially periodic grid-like repeating pattern (Hafting et al., 2005) (Figure 1). Together, these two classes of neurons are thought to provide animals with information about their spatial position in the environment. New work by Giocomo et al. (2011) in this issue of *Cell* and by Hussaini et al. (2011), recently appearing in *Neuron*, elucidate the cellular basis for the unique patterns of neural activity of grid and place cells.

Previous work has shown that both grid and place cells are modulated by ~8 Hz theta rhythm. The theta phase of spikes of place cells contains information about spatial position, called phase precession (O'Keefe and Recce, 1993). Computational models posit that phase precession and grid fields arise through a common mechanism called oscillatory interference (Burgess et al., 2007; Giocomo and Hasselmo, 2009; O'Keefe and Recce, 1993) in which two sources of theta oscillations, an "external" theta, arising from the medial septum, and an "internal" theta, coming from other sources, have slightly different frequencies and thus generate interference patterns.

The internal theta invoked in most computational models has been proposed to result from the hyperpolarization-activated cation current (I_h), which is conducted by HCN channels (Giocomo and Hasselmo, 2009; Nolan et al., 2004). HCN channels are found in many brain regions, including the mEC stellate cells and the hippocampal CA1 pyramidal neurons, especially in their distal dendrites (Nolan et al., 2004). Activation of I_h prolongs the duration of hyperpolarization, thereby suppressing the responsiveness of these neurons to excitatory inputs, especially to low-frequency stimulation. As a result, mEC stellate cells display spontaneous, subthreshold theta modulation in vitro. Further, the frequency of this theta rhythm is lower in the more ventral mEC cells, which could explain, according to oscillatory interference, why their grid